

Several liters of LB + Amp were grown from a pUC18 Y631F SP6 RNA polymerase clone overnight at 37°C. Cells were harvested, lysed and Y631F mutant SP6 RNA polymerase was purified approximately according to standard methods (Butler and Chamberlin, 1983).

Transcription Reactions

To verify that Y631F SP6 RNAP had the desired phenotype, *in vitro* transcription reactions were done where one of the four rNTPs was substituted by the corresponding dNTP (Souza and Padilla, 1995). As expected, the Y631F SP6 RNAP mutant displayed reduced dNTP/rNTP discrimination compared with wild-type SP6 RNAP, similar to that observed for the Y639 mutant of T7 RNAP.

In a standard *in vitro* transcription reaction using the four ribonucleoside triphosphates (rATP, rGTP, rCTP, and rUTP), both enzymes, the wild-type SP6 RNA polymerase as well as the Y631F mutant, synthesized the correct 1.4 kb transcript and in the expected amounts as visualized on gels. However, if one of the four ribonucleoside triphosphates, rGTP for example, is completely substituted by dGTP and *in vitro* transcription reactions are done with the wild-type and mutant enzymes, no transcript is made by the wild-type enzymes. However, the mutant enzyme makes the expected full length transcript in good yield as observed on agarose gels..

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Sousa, Rui
Jendrisak, Jerome J.

(ii) TITLE OF INVENTION: METHODS FOR USING MUTANT RNA POLYMERASES WITH
REDUCED DISCRIMINATION BETWEEN NON-CANONICAL
AND CANONICAL NUCLEOSIDE TRIPHOSPHATES

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Milwaukee
(D) STATE: Wisconsin
(E) COUNTRY: U.S.A.
(F) ZIP: 53202-4497

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Baker, Jean C.
(B) REGISTRATION NUMBER: 35,433
(C) REFERENCE/DOCKET NUMBER: 310307.90067

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (414) 277-5709
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAGACCGG AAU

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGAAATTAAT ACGACTCACT ATA

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGGGGGGGG GACT

14

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGACACGGCG AA

12

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCGGGATGG AATGGAGTAT TCGCCGTGTC CATGGCTGTA AGTATCC

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